Brief Report

Rituximab immunotherapy results in the induction of a lymphoma idiotype-specific T-cell response in patients with follicular lymphoma: support for a “vaccinal effect” of rituximab

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Running Head: Lymphoma specific T-cell responses after Rituximab

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Abstract

The incorporation of rituximab, a chimeric anti-CD20 monoclonal antibody, into the therapeutic armamentarium for patients with follicular lymphoma (FL) has significantly improved treatment outcome for such patients. Despite the almost universal application of this therapy however, its exact mechanism of action has not been completely defined. One proposed mechanism is that of a “vaccinal” effect, whereby FL cell kill by rituximab results in the elicitation of a FL specific T-cell response. The demonstration that rituximab can even elicit such a response in patients, has to our knowledge, never been shown. We analyzed the response against the immunoglobulin expressed by the FL before and after rituximab monotherapy in five FL patients and found an increase in FL idiotype specific T-cells post-rituximab in 4/5 patients. Our data thus provide “proof of principle” for the ability of passive immunotherapy with rituximab to elicit an active FL specific cellular response.
Introduction

Rituximab, a chimeric anti-CD20 monoclonal antibody, has shown significant clinical activity in patients with follicular lymphoma (FL). The proposed mechanisms by which rituximab directly kills FL cells in vivo are complement dependent cytolysis, antibody dependent cellular cytotoxicity or direct induction of apoptosis, although the contribution of each in mediating the clinical effects of rituximab are not well defined. In addition, there has been much recent speculation that rituximab has a 'vaccinal effect', eliciting an active immune response so to account for the ongoing clinical benefit often seen after a single treatment course. However, to date, there has been no evidence that rituximab treatment elicits such a lymphoma specific T-cell response in patients with FL.

To determine whether rituximab treatment elicits a lymphoma specific T-cell response, the frequency of lymphoma idiotype specific T-cells (Id-T-cells) prior to (within several weeks of the 1st dose with no intervening therapy), and approximately one month after the last dose of rituximab, was studied in five patients with FL.

Methods

Patients

Patients with FL treated with single agent rituximab (375 mg/m² weekly X 4 weeks) underwent blood drawing (patients 1 and 5), or leukapheresis (patients 2-4), as well as a lymph node biopsy under a University of Rochester Institutional Review Board approved protocol. Informed consent was obtained in accordance with the Declaration of Helsinki.

Generation of idiotypic protein (Id)

Each Id was generated from the node biopsy by Favrille Inc., as previously described.

In vitro Stimulation (IVS) and ELISpot

Monocyte derived dendritic cells were generated from pre-rituximab Peripheral Blood Lymphocytes (PBL) in Aim-V serum free media, pulsed with autologous patient derived Id protein, matured overnight and then used to stimulate pre- or post-treatment lymphocytes for 1 week (Patients 1 and 5), or 2 weeks (patients 2-4), as previously described. The resultant effector cells were rested overnight then restimulated in IFN-γ ELISpot plates with mature DC (DC), DC pulsed with either the patient’s lymphoma-specific Id (DC-Id) or an irrelevant Id (DC-irr; derived from a different patient) and the number of IFN-γ secreting cells for each condition was determined according to the manufactures recommendations (Mabtech AB, Sweden).

Statistical analysis

The primary endpoint was the number of IFN-γ secreting T-cells. The study design included three experimental conditions (DC; DC-Id; DC-irr) and two time points (pre- and post-rituximab). For each condition and time point, the primary endpoint was measured in triplicate. Two-way mixed ANOVA models were used to describe the primary endpoint. This model included a random intercept for each patient, as well as condition, time, and their interaction as factors. Hypothesis testing was conducted using likelihood ratio tests.
Results and discussion

Patient characteristics are shown in Table 1. As shown in Figure 1A, for patients 1, 2, 3 and 5 there is an increase in the number of IFN-γ secreting cells upon restimulation of 1 week (patients 1 and 5), or 2 week (patients 2 and 3) IVS effector cells with DC-Id as compared to that seen with either DC or DC-irr in the post-rituximab treated PBL. This was not the case for patient 4 at 2 weeks, however. In contrast, the number of IFN-γ secreting cells from the pre-rituximab-PBL showed either no or minimal Id-T-cell responses in all 5 patients. Enough cells were available to repeat the studies for patients 2, 3 and 4, and similar results were obtained (data not shown). As noted in Table 1, patient 2 received a GM-CSF-Id-KLH vaccine (using the “vac-Id”) prior to this study. The patient was then treated with rituximab, and the analysis on the pre- and post-rituximab PBL was conducted using Id’ (Figure 1A). To ensure that the response observed in this IVS was due to a rituximab elicited response to unique determinants of Id’ and not to determinants shared with the vac-Id, the same experiment was conducted using the vac-Id in the 2 week IVS. As shown in Figure 1A (Patient 2 vaccine Id panel), a vac-Id specific response was seen pre-rituximab, however there was no increase in such a response post-rituximab, when using either the vac-Id or Id’ for restimulation, which would have been expected if rituximab was eliciting responses directed against shared determinants with the vac-Id.

If rituximab elicits a lymphoma specific Id-T-cell response, then the number of IFN-γ secreting cells following stimulation with; (a) DC-Id would be greater than DC in the post-rituximab samples as compared to the pre-rituximab samples, therefore the difference in the number of IFN-γ secreting cells between DC-Id and DC in the post-rituximab samples minus the difference in the number of IFN-γ secreting cells between DC-Id and DC in the pre-rituximab samples (the difference of the difference), would be greater than zero; (b) DC-Id would also be greater than DC-irr in the post-rituximab samples as compared to the pre-rituximab samples, and would therefore also be greater then zero, whereas; (c) DC and DC-irr in both the pre- and post-rituximab samples would be the same, and thus would be centered around zero. In Figure 1B, the data are presented showing the difference of the difference between the numbers of IFN-γ secreting cells stimulated by DC and DC-irr; DC-Id and DC; and DC-Id and DC-irr, pre- and post-rituximab for each patient. The data from patients 1, 2, 3 and 5 meet the above criteria whereas the data from patient 4 do not (Figure 1B, symbols). Data from all 5 patients were pooled (displayed as bars in Figure 1B) and a two-way mixed ANOVA, accounting for intra-patient correlation, was used to assess statistical significance. Using this analysis we show that there was a significantly greater response elicited by DC-Id, as compared to that of DC, in the post-rituximab samples, as compared to the pre-rituximab samples (p=0.017). In addition, there was a significantly greater response elicited by DC-Id, as compared to that of DC-irr, in the post-rituximab sample, also as compared to the pre-rituximab samples (p=0.016). In contrast there was no statistically significant difference between the responses to DC vs. DC-irr when comparing the pre- and post-rituximab samples (p= 0.92). To confirm that the conclusions were not complicated by the prior Id vaccination of patient 2, the statistical analysis was performed excluding this patient, and the results remained significant (p<0.05). Taken together, these data provide proof of principle that rituximab can elicit an Id-T-cell response in patients with FL.

The rationale as to why rituximab might elicit an active cellular immune response is that regardless of its mechanism of action, rituximab treatment likely results in the inflammatory death of lymphoma cells. This would result in a depot of tumor antigens released in the context of a microenvironment favoring the uptake, processing and presentation of tumor associated antigens by DCs to T-cells, resulting in the elicitation of a cell-mediated lymphoma specific
immune response. Finally, the engagement of DC activating FcγRs by rituximab may further induce DC activation and maturation, thus favoring the generation of a cellular immune response.  

In our study, there was no obvious association between the clinical response to rituximab and the elicitation of an Id-T-cell response. This is not surprising as there are likely multiple interacting mechanisms which determine clinical response. In addition, it is most probable that rituximab also elicits T-cell responses to lymphoma specific antigens other than the idiotype, which were not captured in this study. Our study does show for the first time however, that passive immunotherapy with rituximab results in the induction of an active cellular response directed against a lymphoma associated antigen. To determine if this is in fact, a bone-fide mechanism of action of rituximab will require an analysis of a larger number of patients as well as the correlation of immune responses to clinical responses.

The demonstration that rituximab generates an active lymphoma specific immune response could have profound implications as to how we optimally treat patients with FL. Specifically, if the elicitation of a lymphoma Id-T-cell response having immunological memory can be augmented, it is possible that both the progression free and overall survival after rituximab may be prolonged. Given that the Id specific responses shown in this study were not very robust, to elicit such clinically relevant responses, rituximab treatment will need to be coupled with strategies designed to overcome the FL induced inhibition of the immune response. Consistent with this assumption, patient 5 had the most robust response and had a very low tumor burden, which is typically thought to be associated with less tumor-associated immune suppression. We and others have shown that one such mechanism of FL-associated immune suppression is increased numbers of Tregs within the FL microenvironment. The challenge will be to overcome these and other mechanisms of tumor induced immune suppression.

Acknowledgements

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Authorship

Contributions: S.P.H. designed research, performed research, analyzed data and wrote the paper; O.H. performed statistical analysis and wrote the paper; T.R.M., A.M.L., J.W.F., F.Y., R.I.F., R.J.K., R.B.B. and S.H.B. designed research, analyzed data and wrote the paper.

References


Table 1. Patient characteristics.

<table>
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<th>Patient</th>
<th>Pathology</th>
<th>Prior treatment</th>
<th>Rituximab response</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Follicular, grade 2</td>
<td>none</td>
<td>CR</td>
</tr>
<tr>
<td>2</td>
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<td>Rituximab, GM-CSF-Id-KLH vaccine*</td>
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<td>3</td>
<td>Follicular, grade 3</td>
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</tr>
<tr>
<td>5</td>
<td>Follicular, grade 1</td>
<td>none</td>
<td>PR</td>
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* At the time of relapse and rituximab re-treatment, a new dominant clone using a different Id (Id') than that used for the Id vaccine emerged. This new Id (Id') was used in the current study. GM-CSF: Granulocyte-Macrophage Colony Stimulating Factor; Id-KLH: Idiotype-Keyhole Limpet Hemocyanin; DLBCL: diffuse large B-cell lymphoma; R-CHOP: Rituximab, Cyclophosphamide, Adriamycin, Vincristine, Prednisone; APBSCT: Autologous Peripheral Blood Stem Cell Transplant; IFXRT: Involved Field Radiation Therapy; CR: complete response; PR: partial response; PD: progressive disease.
**Figure 1.** Rituximab treatment elicits a lymphoma idiotype-specific IFN-γ T-cell response. Immature DC from all 5 patients were generated, pulsed with autologous patient lymphoma derived idiotype protein (Id) and matured overnight. Pulsed/matured DC were then used to stimulate pre- or post-rituximab treatment lymphocytes for 1 week (patients 1 and 5), or 2 weeks (patients 2, 3 and 4) in AIM-V serum free media with IL-2. The resultant week 1 or 2 effectors were then harvested, washed and rested overnight in AIM-V media containing IL-2. Rested effectors were stimulated in IFN-γ ELISpot plates (in triplicate) with mature DC alone (DC) or DC pulsed and matured (as above) with either the patient’s lymphoma specific Id (DC-Id) or an irrelevant Id derived from a different patient (DC-Irr). The number of IFN-γ secreting cells for each condition was then determined by standard ELISpot methods after incubation overnight. (A) The number of IFN-γ spots for patients 1 through 5, for both pre- or post-rituximab effectors stimulated overnight by: DC; DC-Id; DC-Irr are shown, as is the IVS repeat for patient 2 using the vaccine specific Id for the IVS rather than the relapse Id. (B) The difference between the numbers of IFN-γ secreting cells upon stimulation with: DC vs. DC-Irr; DC-Id vs. DC; DC-Id vs. DC-Irr was calculated for each of the pre- and post-Rituximab samples (eg: (DC-Id - DC)\text{Post}) and then the difference between the pre- and post-rituximab samples was calculated (eg: (DC-Id - DC)\text{Post} - (DC-Id - DC)\text{Pre}). A final difference that is greater in the post-rituximab sample, as compared to the pre-rituximab sample, is plotted as a positive number (above zero), whereas a difference that is similar between the pre- and post-rituximab samples is plotted around zero. The data are shown for each individual patient (symbols), as well as for the averages computed over all 5 patients for each condition (bars), with the corresponding p-values from tests assessing the significance of each bar.
**A**

Patient 1

Patient 2

Patient 3

Patient 4

Patient 5

**B**

\(p=0.92\)

\(p=0.017\)

\(p=0.016\)
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